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Prolonged Chronic Graft-versus-Host Disease is a Risk Factor for Thyroid Failure in Long-Term Survivors After Matched Sibling Donor Stem Cell Transplantation for Hematologic Malignancies

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We studied thyroid function in 81 long-term survivors of allogeneic stem cell transplantation (allo-SCT), with a median follow-up of 84 months (range, 45 to 166 months). Median age at transplantation was 35 years (range, 6 to 66). Seventy-two of the patients received a total body irradiation (TBI)–containing conditioning regimen (n = 23, 12 Gy; n = 49, 13 Gy). Twenty-one of the patients (25.9%) had subclinical hypothyroidism, and 9 (11.1%) developed overt hypothyroidism at a median of 28 months (range, 3 to 78 months) after allo-SCT. Multivariate logistic regression analysis demonstrated that prolonged immunosuppressive therapy (IST) was significantly associated with subclinical hypothyroidism (odds ratio [OR] = 3.8) and overt hypothyroidism (OR = 2.6). Antithyroglobulin and thyroid peroxidase antibody were detected in 12 of 60 patients tested (20%). No correlation was found between the occurrence of thyroid antibodies and hypothyroidism (P = .13) or chronic graft-versus-host disease (cGVHD) (P = .55). In conclusion, thyroid dysfunction is relatively common after allo-SCT and is more likely to occur in patients receiving prolonged IST for cGVHD; however, thyroid dysfunction does not appear to be related to an antibody-mediated autoimmune process.

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INTRODUCTION

Nearly 90% of patients alive 2 years after allogenic stem cell transplantation (allo-SCT) will become long term survivors [1], shifting the focus of care from cure of the original disease to the identification and treatment of delayed and long-term complications that may affect quality of life [2-4]. Thyroid failure (both overt and subclinical) is a recognized long-term complication after allo-SCT that can affect quality of life and predispose to cardiac and metabolic complications [5]. Approximately 15% of surviving patients are reported to develop hypothyroidism, and twice that number (30%-40%) develop compensated hypothyroidism [6-8]. Time to onset of hypothyroidism after allo-SCT is reported to vary between 1 year and 10 years [6-8]. Thyroid failure has been linked to total body irradiation (TBI), but also has been noted after non-TBI conditioning regimens [7,8]. As transplantation centers increasingly use non-TBI conditioning (with inclusion of older adults), it is becoming more important to define risk factors for thyroid dysfunction other than the conditioning regimen. Although a few studies have linked thyroid dysfunction with chronic graft-versus-host disease (cGVHD), no consistent link has been demonstrated; furthermore, a relationship among antithyroid antibodies, cGVHD, and thyroid dysfunction has not been clearly established [9].

In this work, we studied thyroid function in patients surviving more than 3 years posttransplantation. Our aim was to identify factors associated with hypothyroidism and to explore the relative contributions of conditioning regimen, autoantibodies, and cGVHD to thyroid dysfunction.

METHODS

A total of 417 patients with hematologic disorders underwent allo-SCT from an HLA-identical sibling

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between 1993 and 2003. Patients at a minimum of 3 years posttransplantation were enrolled between April 2005 and October 2006 in an institutional review board–approved long-term evaluation protocol (NHLBI 05-H-0130; ClinicalTrials.gov identifier NCT00106925). Of the 111 patients surviving 3 or more years at the time of study initiation in 2005, 84 patients gave written informed consent according to principles outlined in the Declaration of Helsinki. Thyroid function tests were performed in 81 of these patients. Transplantation conditioning consisted mainly of TBI (12 to 13.6 Gy) and cyclophosphamide (Cy; with and without fludarabine [Flu]) (n = 72) or reduced-intensity conditioning (RIC) with Flu and Cy (n = 9). All patients received cyclosporine (CsA) as GVHD prophylaxis. Acute GVHD (aGVHD) and cGVHD were graded based on published criteria [10,11]. Patient characteristics are summarized in Table 1.

Thyroid Function Tests and Definitions

Thyroid-stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3) levels were measured before transplantation and then at 3 months, 6 months, and annually after transplantation. Earlier and more frequent measurements were made when clinically indicated. In the last 60 patients studied, antithyroid antibodies (antithyroglobulin and thyroid peroxidase antibody) also were measured.

Subclinical hypothyroidism was defined as elevated TSH with normal T4 and T3 levels in asymptomatic patients, with overt hypothyroidism defined as elevated TSH with low T4 and/or T3 levels with or without clinical features. In each patient hypothyroidism was defined as subclinical or overt, whichever occurred first in this analysis.

Statistical Analysis

Clinical and transplantation characteristics were used to evaluate risk factors for hypothyroidism. The variables included in the analysis are listed in Table 2. In univariate analyses, χ^2 or Fisher's exact tests were used for categorical variables, and the Mann–Whitney U-test was used for continuous variables. Summary statistics (eg, proportions, means, standard deviations, 95% confidence intervals, medians, and ranges) were used to describe the patient characteristics, pretransplantation variables, and posttransplantation outcomes. Kaplan–Meier curves were used to display the distributions of events among subgroups of patients. Logistic regression was used for multivariate analysis of associated variables. Statistical significance was accepted at $P < .05$. All data analyses were performed using SPSS 15 for Windows (SPSS Inc, Chicago, IL).

Table 1. Patient Characteristics*

Variable (n = 81)	n (%)
Age at transplantation, years, median (range)	35 (6 to 66)
Sex, n (%)	
Male	47(58)
Female	34 (42)
Disease distribution, n (%)	
Acute myelogenous leukemia/myelodysplastic syndrome	25 (30.8)
Acute lymphocytic leukemia	3 (3.7)
Chronic myelogenous leukemia	49 (60.5)
Others	4 (4.9)
Stem cell source, n (%)	
Bone marrow	15 (18.5)
Peripheral blood stem cells	66 (81.5)
Conditioning regimen, n (%)	
TBI–based myeloablative conditioning	72 (90)
Reduced-intensity conditioning	9 (10)
Acute GVHD, n (%)	
Grade I-IV	35 (43.2)
Grade II-IV	15 (18.5)
Chronic GVHD, n (%)	66 [limited, 50; extensive, 16] (81.5)
Prolonged IST (> 3 years), n (%)	14 (17)
Mortality beyond 3-year follow-up, n (%)	2 (2.5)
Hypothyroidism (time to hypothyroidism, median and range), n (%)	
All cases (28 months; 3 to 78 months)	30 (37)
Subclinical (20 months; 3 to 38 months)	21 (25.9)
Overt (32 months; 9 to 78 months)	9 (11.1)
Total patients on treatment, n (%)	20 (24.7)
Positive antithyroid antibodies (n = 60), n (%)	12 (20)

GVHD indicates graft-versus-host disease; TBI, total body irradiation; IST, immunosuppressive therapy.
*Follow-up period, median of 84 months (range, 25 to 166 months).

RESULTS

Table 1 summarizes patient characteristics, and Table 2 reports the results of univariate analysis of factors associated with subclinical and overt hypothyroidism. Hypothyroidism occurred in 30 patients, with a cumulative incidence in these 3-year survivors of 40% ± 5.9% (Figure 1). The median time to onset of hypothyroidism was 28 months (range, 3 to 78 months), 20 months (range, 3 to 38 months) for the subclinical form and 32 months (range, 9 to 78 months) for the overt form. Hypothyroidism was more likely to occur in older patients (Table 2; Figure 2A) and in patients requiring prolonged immunosuppressive therapy (IST) for long-standing cGVHD (Table 2; Figure 2B, C, D).

To further explore the relationships among cGVHD, prolonged IST, and hypothyroidism, we analyzed the cGVHD group separately. A total of 52 patients developed cGVHD requiring treatment for less than 3 years. Of these, 13 (25%) developed hypothyroidism, compared with 11 of 14 patients (78.5%) with cGVHD persisting beyond 3 years and requiring IST ($P < .0001$). Our data demonstrate no significant difference in the rate of hypothyroidism ($P = .088$) and positive thyroid antibodies ($P = .5$) between patients with limited cGVHD and those with extensive cGVHD. Thus, the duration of cGVHD appears to

Table 2. Factors Associated with Hypothyroidism

	Subclinical		Overt		Overall	
Factor (n)	n (%)	P value	n (%)	P value	n (%)	P value
Age (< vs > median)		.526		.484		.557
≤ median (41)	11 (26.8)		4 (9.7)		15 (36.5)	
> median (40)	10 (25)		5 (12.5)		15 (37.5)	
Age quartiles		.161		.037		.476
First three (61)	18 (29.5)		4 (6.5)		22 (36)	
Oldest quartile (20)	3 (15)		5 (25)		8 (40)	
Sex		.193		.428		.335
Male (47)	10 (21.2)		3 (8.8)		16 (34)	
Female (34)	11 (32.3)		6 (12.7)		14 (41.1)	
Type of SCT		.413		.528		.494
Bone marrow (15)	3 (20)		2 (11.7)		5 (33.3)	
Peripheral blood stem cells (66)	18 (27.2)		7 (10.6)		25 (37.8)	
TBI*		.573		.261		.442
Yes (72)	19 (26.3)		7 (9.7)		26 (36.1)	
No (9)	2 (22.2)		2 (22.2)		4 (44.4)	
Acute GVHD		.184		.214		.494
Grade 0-I (66)	19 (28.7)		6 (9)		25 (37.8	
Grade II-IV (15)	2 (13.3)		3 (20)		5 (33.3)	
Chronic GVHD		.192		.432		.365
Yes (66)	15 (23)		8 (12.1)		23 (34.8)	
No (15)	6 (40)		1 (6.7)		7 (46.6)	
Prolonged IST†		.031		.044		.001
Yes (14)	7 (50)		4 (28.5)		11 (78.5)	
No (67)	14 (20.8)		5 (7.4)		19 (28.3)	

SCT indicated stem cell transplantation; TBI, total body irradiation; GVHD, graft-versus-host disease; IST, immunosuppressive therapy.
*No significant difference in patients with a TBI dose of 12 Gy and those with a TBI dose of 13.6 Gy.
†IST beyond 3 years posttransplantation.

be a more important factor than occurrence or severity of cGVHD. These data must be interpreted with caution, because only a small number of patients developed extensive cGVHD. More than half of the patients with subclinical hypothyroidism (11 of 21) eventually required thyroid replacement therapy. Multivariate logistic regression analysis showed that prolonged IST was independently associated with both overt and subclinical hypothyroidism (overt: odds ratio [OR] = 2.6, 95% confidence interval [CI] = 1.1 to 21.4, $P = .04$; subclinical: OR = 3.8, 95% CI = 1.2 to 14.4, $P = .03$). In the subgroup of 11 patients receiving prolonged IST initially diagnosed with subclinical hypothyroidism, all became symptomatic and required thyroid replacement therapy.

Relationships among Antithyroid Antibodies, cGVHD, and Hypothyroidism

Sixty of the 81 patients were tested for antithyroid antibodies (antithyroglobulin and thyroid peroxidase antibody), and 12 (20%) were positive for either or both antibodies. But no significant association was found between the presence of antithyroid antibodies and hypothyroidism; antibodies were found in 7 of 24 patients (29%) with hypothyroidism and in 5 of 36 (14%) without hypothyroidism ($P = .13$). Similarly, no association was found between cGVHD and positive antithyroid antibodies; 10 of 48 patients (20.8%) with a history of cGVHD were positive, compared with 2 of 12 (16.6%) without cGVHD ($P = .55$) (Table 2). To further explore the relationship between prolonged IST and antithyroid antibodies, we analyzed patients with history of cGVHD separately. Four of 12 patients (33%) requiring prolonged IST (> 3 years) for cGVHD were positive for antithyroid antibodies, compared with 6 of 36 (17%) with cGVHD of less than 3 years duration ($P = .202$). These results further confirm a lack of association between antithyroid antibodies with cGVHD requiring prolonged IST.

DISCUSSION

Our findings demonstrate that hypothyroidism is a significant late complication in long-term allo-SCT survivors, occurring in almost 40% of patients surviving 3 or more years after transplantation SCT. In contrast to previous studies [7,12-15], we found prolonged IST for ongoing cGVHD (both overt and subclinical hypothyroidism) and increasing age (overt hypothyroidism) as the only risk factors for hypothyroidism in patients followed up for a median of 7 years.

Previous studies of thyroid function tests in children and adolescents receiving allo-SCT showed that younger children (aged < 10 years) were more likely to have thyroid dysfunction compared with older children [6,16]. Our studies do not contradict these observations, because it included only 2 patients aged < 10 years at the time of transplantation; most of the patients were adults, including 21 patients aged > 44 years. The patients aged 44 to 66 years had

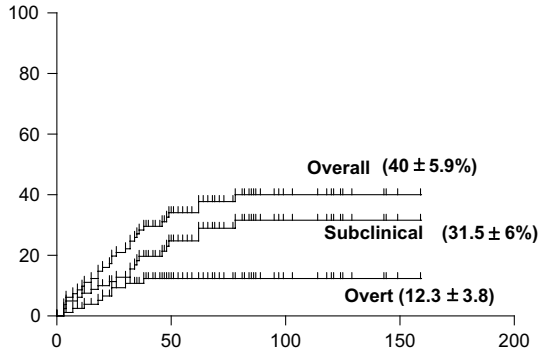

Figure 1. Cumulative incidence of hypothyroidism.

a significantly higher rate of overt hypothyroidism in univariate analysis; however, the effect of age was confounded by the fact that 8 of 14 patients in this older cohort received prolonged IST for cGVHD.

Although no significant impact of conditioning regimen intensity (Table 2) was found, we cannot exclude a possible contribution of TBI to the development of hypothyroidism, because only a minority of our patients received a nonmyeloablative conditioning regimen. Our data suggest that the thyroid may be susceptible to alloimmune attack associated with prolonged cGVHD; however, we could not confirm that cGVHD per se is a provocative factor for thyroid damage, because we found no relationship among hypothyroidism, antithyroid antibodies, and cGVHD. It may be that thyroid damage is simply T cell–mediated or that prolonged IST per se damages the thyroid gland. Importantly, we found that all patients with subclinical hypothyroidism requiring prolonged IST developed symptomatic hypothyroidism and required replacement therapy. This might indicate the need for early replacement therapy, especially for those in this patient group who are seen infrequently at our clinic. The debate continues on whether or not to treat patients with subclinical hypothyroidism [6,17,18]. Initially, we and others [6] did not treat subclinical hypothyroidism, in contrast to other investigators [18]. An important rationale for treating subclinical hypothyroidism is to decrease the risk of thyroid adenoma and carcinoma [19,20] and, in young patients, to prevent growth failure and delayed development. SCT recipients are at increased risk of developing second malignancies [21,22]. A European Group for Blood and Marrow Transplantation study found that thyroid cancer was the most common secondary cancer, with a standardized incidence ratio approaching 50 in long-term survivors after SCT. Similar to our findings, the risk factors for developing secondary cancer were extensive cGVHD and IST for cGVHD. To date, none of our patients has developed thyroid cancer or hyperthyroidism.

The development of thyroid dysfunction after allo-SCT has been linked to an autoimmune process; however, the true incidence of clinically significant autoimmune thyroid dysfunction after allo-SCT remains unknown. It has been reported that thyroid damage after allo-SCT, causing transient subclinical hypothyroidism and low-titer thyroid antibodies, may be

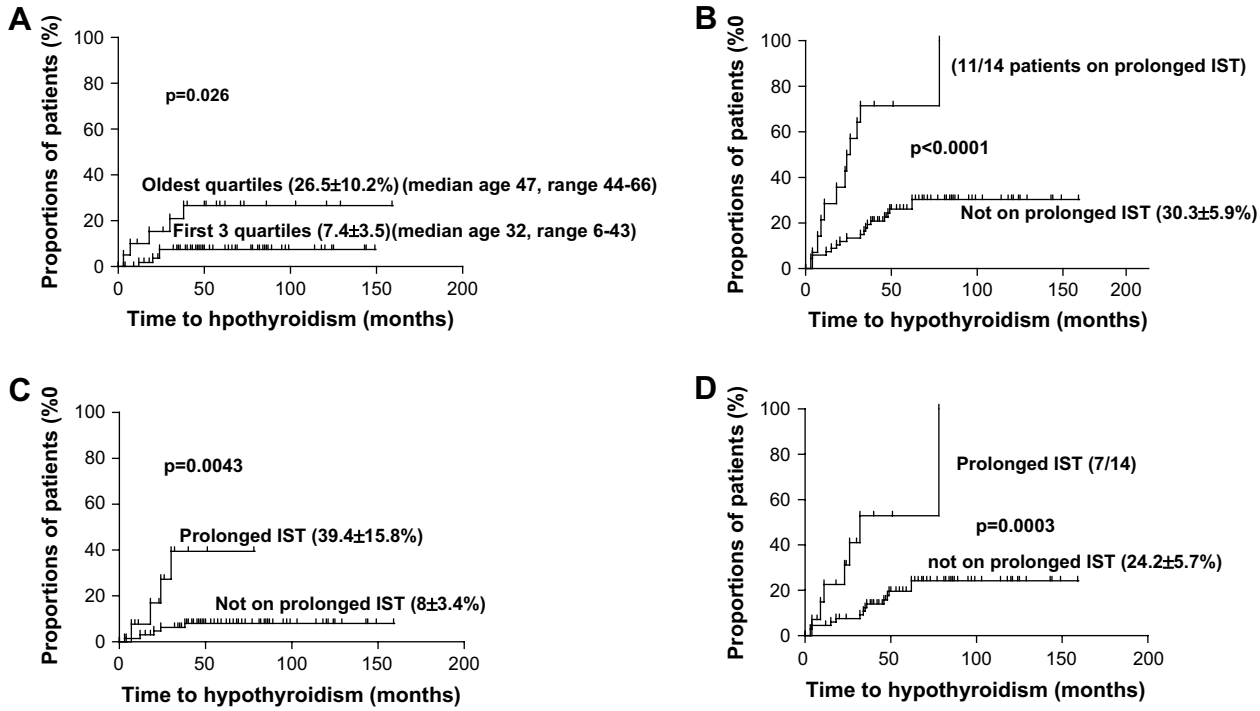

Figure 2. Associations between age and overt hypothyroidism (A), prolonged IST and hypothyroidism (B), and prolonged IST and overt (C) and subclinical (D) hypothyroidism.

common [23]. In small case series, autoimmune thyroid dysfunction has been described in up to 3% of allo-SCT survivors [9,24]. But we found no correlation between the development of thyroid autoantibodies and hypothyroidism. Thus, although an alloimmune response may contribute to thyroid dysfunction after allo-SCT, it does not appear to be mediated through the classical autoantibody pathway. Further investigation is needed to determine how the cGVHD process affects the thyroid.

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